THE EFFECT OF THE PATHOGENIC RHIZOPOD HYDRAMOEBA HYDROXENA (ENTZ) ON REPRODUCTION IN CHLOROHYDRA VIRIDISSIMA UNDER VARIOUS LEVELS OF TEMPERATURE[1]

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Since the discovery by Entz (1912) of *Hydramoeba hydroxena*, which attacks the fresh water polyp hydra, very few attempts have been made to elucidate the ecological and pathological relationships between these organisms; in fact, only those of Reynolds and Looper (1928), Ito (1949, 1950) and Rice (1960) are noteworthy. The ease of culturing large numbers both of host and parasite and the rapidity with which the hydramoebae attack and complete the destruction of many host species suggested a convenient and relatively simple system that would be ideal for studying ecological phenomena associated with population host-parasitism, and for exploring certain epidemiological concepts through controlled experiments. This paper, therefore, is one of a series (Stiven, 1962a; in press) in which this goal has been pursued.

In the analysis of the initiation, progress, and outcome of artificially induced epidemics carried out over several periods of budding in the host population, it is important to know what inhibitory effect the infection has on the host's ability to reproduce, and hence on the reproductive potential and subsequent density of the host population. The relationship between host density and parasite density, and the rate of increase of an epidemic was considered mathematically as early as 1923 by Lotka and later experimentally and mathematically by many others (*e. g.*, Burnett, 1949; Watt, 1959). The consequences of host density and the rate of spread of the infection have also been examined in the hydra-hydramoeba system (Stiven, in press). In this case, however, all buds which detached from parent hydras were immediately removed, thus maintaining the host populations at constant densities throughout any one epidemic. The effect of a changing density of host population through accumulation of buds, and the influence of various key environmental factors on such changing densities have yet to be tested on the rate of spread of the hydramoeba epidemic.

In addition, little is known about the importance of hydramoebae in natural populations of hydra. Hydras apparently undergo seasonal fluctuations in abundance, although relevant studies are rather limited in number (*e.g.*, Welch and Loomis, 1924; Miller, 1936; Bryden, 1952). Miller (1936) reported the presence of the parasite in late summer and early autumn in declining populations of hydras in Douglas Lake, Michigan. Some populations were not noticeably parasitized,

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whereas others were heavily infected. He concluded that *Hydramoeba* might be an important factor in reducing certain populations, but that it is probably unimportant in determining the quantitative seasonal distribution of hydras. Working in Kirkpatricks Lake in Tennessee, Bryden (1952) briefly mentioned that during the summer of 1949 large numbers of hydramoebae appeared in one of his populations of hydras and that within a short time the entire population disappeared, apparently because of the detrimental effects of the parasite.

It was the purpose of this study, therefore, to determine the degree to which different levels (magnitudes) of initial infection with *Hydramoeba* affect the rate of asexual reproduction in *Chlorohydra viridissima* under a range of temperatures compatible with that occurring in their natural environment.

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Materials and Methods

The common green hydra, *Chlorohydra viridissima*, was the principal host in these experiments, although *Hydra pseudoligactis* was used in one experiment. For experimental use, actively budding hydras were maintained in stock cultures at 21° to 23° C. The experimental treatments comprised four levels of temperature, 15°, 20°, 25°, and 30° C., and three levels of initial infection, a light infection of two hydramoebae per hydra, a heavy infection of 8, and a control of uninfected hosts. There were five individual hydras (replicates) for each combination of temperature and initial infection—a total of 60 hydras. Each host, bearing one completely formed bud, was infected and placed in one of the depressions of a 9-depression spot plate; each depression contained approximately 2 cc. of culture medium. The spot plates were kept in sealed moist chambers throughout the experiments. Each individual hydra was fed *Artemia* (brine shrimp) daily as long as it was able to feed. Egestion products were removed four to five hours after feeding, and the culture medium was changed every 24 hours. The medium was a modified tap-water solution developed by Loomis (1953). Constant illumination was maintained with two 15-watt fluorescent lamps placed two feet from the culture dishes. Preliminary experiments indicated that the responses of the host-parasite system of concern to this study occurred within 10 days; consequently, production of buds by each individual hydra was observed for this period starting from the day of initial infection.

The specimens of *Hydra pseudoligactis* were similarly infected, but only one temperature level (25° C.) was used.

Results

Reduction of budding rate in infected C. viridissima

The average number of buds per hydra per day produced by the five replicates under all treatments during the 10 days of the experimental period is depicted in Figure 1. For example, at 20° C. and in the first day of the experiment, the control group produced an average of 1.0 bud per day per hydra, the lightly infected group 0.8, and the heavily infected group 0.6. By the end of the fifth

day the accumulated number of buds per hydra per five days was 4.0, 2.4, 2.0 for the control, light, and heavy infection groups, respectively. By the end of the tenth day these differences had diverged further (8.6, 4.2, and 2.0 buds per

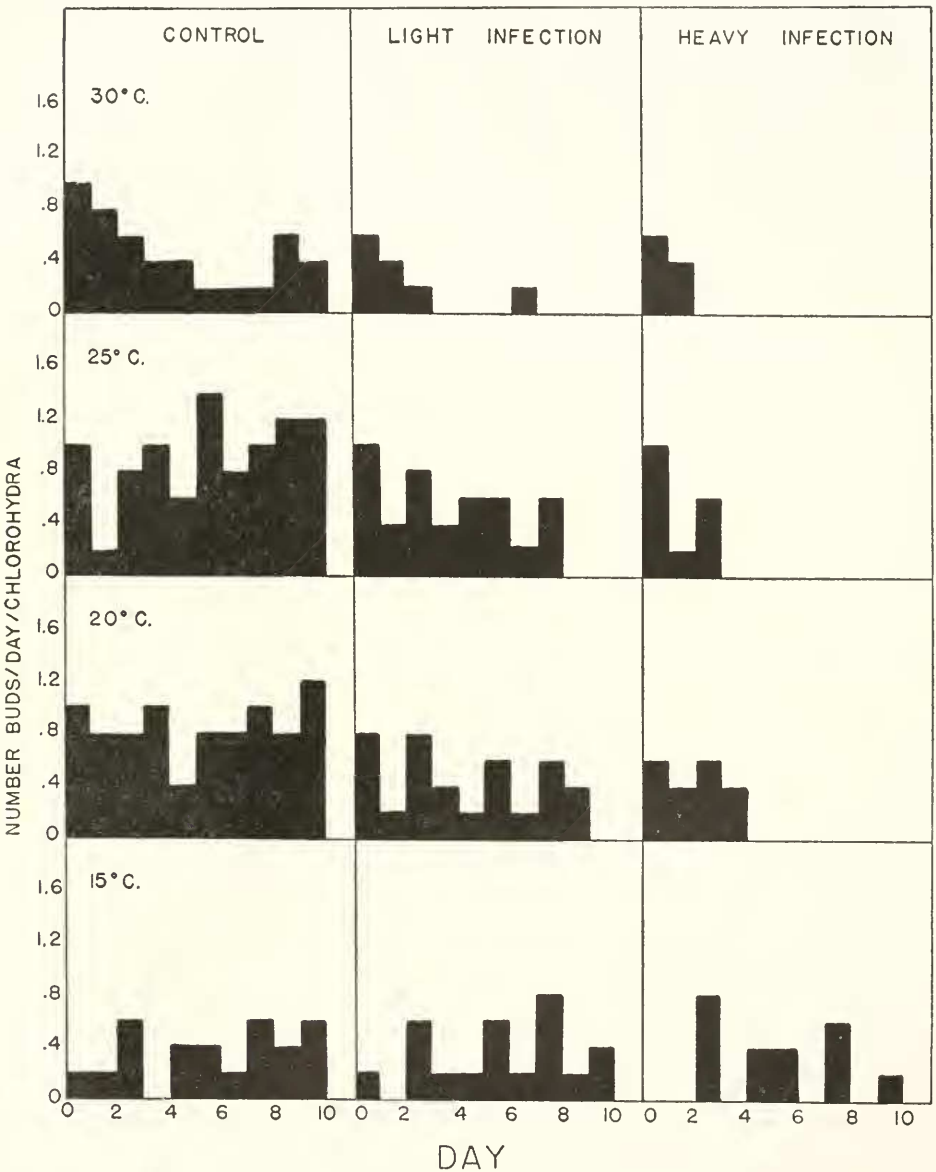

FIGURE 1. Production of buds by infected *Chlorohydra viridissima* during the 10 days of the experiment. Budding rate is represented by the number of buds produced per hydra per day under 4 levels of temperature and 3 levels of initial infection. Each treatment combination consisted of 5 hydras.

hydra per 10 days), since the control group continued to produce buds throughout the entire experiment and the lightly infected group produced them for 9 days.

When the production of buds is compared among the levels of temperature—for example, for the lightly infected group—a sharp drop in bud production occurred at 30° within the first three days of the experiment. At 25° and 20° production of buds also declined, but not as rapidly. At 15°, however, buds were produced throughout the experimental period, but at a lower rate than at 20° and 25° C. This comparison can be expressed quantitatively by summing the daily production of buds for the five replicates of the lightly infected group over the experimental period for the four temperature levels. Thus, the total number of buds per five hydras per 10 days at the respective temperatures (30° through 15°) was 7, 23, 21, and 17. A similar picture is evident for the heavily infected

TABLE 1

Analysis of variance of total bud production by 5 C. viridissima *during the first two days of the experiment under 4 levels of temperature and 3 levels of initial infection. (4 × 3 factorial design)*

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Treatments	11	18.184		
A (Temperature)	3	13.384	4.461	31.5**
B (Infection)	2	3.434	1.717	12.1**
AB Interaction	6	1.366	0.228	1.6 ns
Error	48	6.800	0.142	
Total	59	24.984		

** Significant $P = 0.01$.
ns Not significant at $P = 0.05$.

group. At 30°, bud production ceased by the end of the second day. At 25° and 20°, the production of buds ceased at the end of the third and fourth days, respectively. At 15°, however, the infected hydras continued asexual reproduction throughout the 10-day period.

It is evident that the differences in bud production among the levels of infection and temperature become exaggerated the longer the experiment is carried out, since the controls continue to produce buds, whereas budding in the infected groups either declines or eventually ceases (Fig. 1). The question of importance, therefore, is the significance of the degree of difference in bud production among temperature and infection levels over increasing units of time.

To answer this question, the accumulated number of buds produced during the first two days of the experiment for each replicate under each treatment combination was considered as a simple 4 × 3 factorial experiment. The analysis of variance of these data gives the significance of the effects of temperature and infection, acting alone and in combination (first-order interaction). The results of this analysis are presented in Table 1. The significant main effect, temperature, means that the differences in bud production among the various levels of temperature are statistically significant ($P < 0.05$) when averaged over all levels of initial infection. Similarly, the different levels of initial infection have given rise

to significant differences in bud production during the first two cumulative days of the experiment when averaged over all levels of temperature. The interaction between temperatures and infection, which is not significant, indicates that these two factors are independent; that is, any simple effect of one factor on budding is not dependent upon the level of the other.

It should be noted that no host mortality occurred in any treatment combination during the first two days of this experiment, and that the differences in budding rate, therefore, are due only to the effects of the hydramoebae.

To determine how these two factors continue to affect the production of buds during the remaining cumulative time-intervals of the experiment, similar

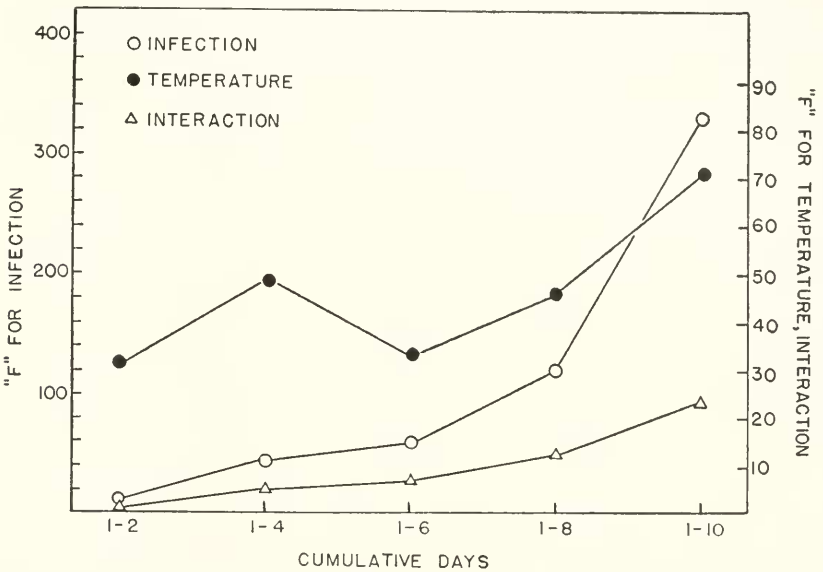

FIGURE 2. Change in the value of F from the analyses of variance of temperature and initial infection effects on bud production. An analysis was performed for each cumulative successive two-day period up to the total of 10 days. The only value of F which is not statistically significant ($P = 0.05$) is the interaction between temperature and infection during the cumulative first two days of the experiment.

analyses of variance were carried out on total bud production by each host for each subsequent cumulative two-day period; that is, from the start of the experiment to the end of the fourth day, from the start to the end of the sixth day, and so on, to the end of the tenth day. This procedure involved four more analyses. The changes in the magnitude of the differences in the effects of the two factors acting separately and in combination are represented by the values of F and are given in Figure 2. Both temperature and infection continued to produce significant effects on the production of buds when examined as single factors. In fact, the degree of significance for infection increases rapidly over time. This was probably due to the early cessation of budding in the heavily infected group. While the interaction was not significant during the first two days

of the experiment, significance appeared in the time-interval of one to four days, and gradually increases up to the tenth day. After the second day, therefore, dependence appeared between these two factors, since the difference in bud production among the levels of temperature differed among the three levels of infection beyond day 2. The converse is also true, since an interaction is symmetrical.

If the production of buds is examined only between the light and the heavy infection levels under the four levels of temperature for the cumulative 10-day period (the control group is omitted from the calculations), these two factors and their interaction continue to affect significantly the production of buds ($P < 0.01$), but with a reduction in the value of F in all cases. A clearer picture of this can be derived by examining the contribution of the control group to the variation attributable to temperature and infection. This is done by analyzing

TABLE II

Proportion of variance attributable to initial infection, temperature, and their interaction when control is included, and excluded, from the analysis of variance. Data for the accumulated 10-day period

Source of variation	Control included		Control excluded	
	Sum of squares	Proportion of variation	Sum of squares	Proportion of variation
A (Temperature)	82.19	0.1990	26.60	0.3715
B (Infection)	255.24	0.6180	25.60	0.3575
AB Interaction	57.16	0.1384	9.00	0.1258
Error	18.40	0.0446	10.40	0.1452
Total	412.99	1.0000	71.60	1.0000

the sums of squares and is presented in Table II. When the control is included, over half (62%) of the total variation is caused by initial infection, but when the control is omitted, temperature and infection each contribute approximately one-third to the total variation. In other words, approximately a 25% decrease in the variation attributable to initial infection, and a 17% increase attributable to temperature have occurred. While the control group does influence greatly the significance of the two factors, its removal from the analysis does not alter the significance of temperature and initial infection within the 95% probability bounds.

In the preceding analyses the interpretation of the changes in budding rate, represented by the changes in the value of F, is complicated by the fact that after the first two days of the experiment host mortality begins to occur in some of the treatments. This is particularly true at the heavy infection level and at the higher temperature (see Figure 1, and Table III, last column). Obviously, the death of one or several hosts in a particular treatment combination will contribute sizeably to the reduction of the budding rate in that treatment. It becomes very difficult to analyze the complete factorial experiment using only those hosts which have survived to the end of the experiment, since in some treatments all hosts succumbed (see Table III), and the factorial design is destroyed. However, it

is possible to analyze the differences in bud production between the light and heavy infection levels for each temperature separately, using only the cumulative time period in which no hosts succumbed. For example, at 15° C. where all hosts survived the entire 10 days, a significant difference ($P < 0.05$) exists between the two infection levels in the mean number of buds per hydra per 10 days. The same result exists at 20° for the first six-day period (hosts started dying after the sixth day in the heavy infection level). On the other hand, at 25° and 30°, no difference exists between the levels of infection in the buds produced per hydra per four days and per two days, respectively. It must be concluded, therefore that the differences in bud production among the levels of infection and

TABLE III

Certain features of the host-parasite relationship which resulted from the initial infection and contributed to the decline and cessation of budding. Values are the mean ± the standard error. The values in brackets indicate the number of hydras participating in the calculation of the means

Treatment		Day host stopped feeding	Days host did not feed	Day last bud produced	Day of death of host
Light infection	15°	—	—	—	—
	20°	7.6 ± 1.07 (5)	1.3 ± 0.58 (3)	8.4 ± 0.24 (5)	10.0 ± 0.00 (2)
	25°	6.6 ± 0.68 (5)	1.8 ± 0.48 (4)	7.2 ± 0.58 (5)	10.0 ± 0.00 (1)
	30°	3.0 ± 0.00 (5)	3.0 ± 0.00 (1)	1.6 ± 0.40 (5)	5.5 ± 0.29 (4)
Heavy infection	15°	—	1.0 ± 0.95 (5)	—	—
	20°	5.0 ± 1.10 (5)	4.0 ± 1.41 (2)	3.4 ± 0.22 (5)	8.0 ± 1.00 (3)
	25°	4.2 ± 0.20 (5)	—	2.4 ± 0.40 (5)	5.8 ± 0.20 (5)
	30°	2.2 ± 0.20 (5)	—	1.4 ± 0.24 (5)	3.6 ± 0.40 (5)

temperature are due solely to the inhibitory effect of the hydramoebae only up to the tenth, sixth, fourth, and second days for 15° through 30°, respectively. Beyond these days the reduction in budding is the result of the combination of host mortality and the hydramoebae, with host mortality playing an increasingly important role as temperature increases up to 30°. Host mortality, of course, is due directly to the action of the hydramoebae.

Reduction of budding rate in infected Hydra pseudoligactis

As already indicated, observation of budding rate in infected individuals of this species was made only at one temperature level, 25° C. The results are graphed in Figure 3. This species, which has a lower level of resistance to hydramoebae than *C. viridissima* (Stiven, 1962a), ceases budding shortly after infection. Comparing the responses of both species at 25° C. we find almost identical numbers of buds produced by the control groups (47 and 49 buds per 5 hydras per 10 days for *C. viridissima* and *H. pseudoligactis*, respectively). It was shown in an earlier study (Stiven, 1962b) that the former species has a slightly higher ($P < 0.05$) intrinsic rate of increase than *H. pseudoligactis* at 25° C. In fact, at 25° and under daily feeding, a green hydra after 10 days detachment from the parent produces an average of 1.61 buds per day, compared with

a value of 1.36 for the brown species. In this experiment, under a light initial infection totals of 22 and 16 buds were produced by the five green and five brown individuals, respectively. Only one green hydra died under this treatment, but all *H. pseudoligactis* individuals succumbed (the average day of death was 6.6). Under a heavy initial infection, identical numbers of buds were produced by both species and the average day of death was 6.2 and 5.4 for the green and brown hydras, respectively. It appears, therefore, that under a heavy initial infection the inherent differences in the level of resistance to the hydramoebae of these two host species are masked.

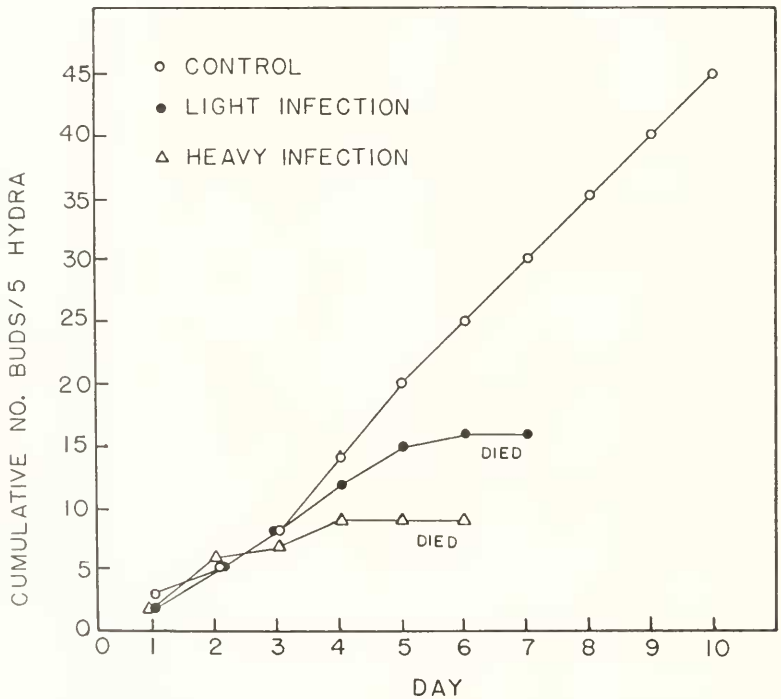

FIGURE 3. Total cumulative production of buds by 5 *Hydra pseudoligactis* under three levels of initial infection. Temperature was 25° C. The mean day of death occurred between the 5th and 6th day and the 6th and 7th day for heavily and lightly infected hosts, respectively.

Mechanisms contributing to reduction in budding

From the experiments employing *C. viridissima*, there is available a certain amount of information which may reveal the nature of the mechanisms leading to a differential decline and cessation of budding. When a host becomes infected, the hydramoeba population first increases to a certain level; next, it either stabilizes or fluctuates slightly; then, just prior to and during the disintegration of the host, a final increase occurs (Stiven, in press). During the attack the tentacles are first consumed. Their loss renders the hydra incapable of feeding, and thus budding ceases. If the infection is severe, death usually follows. In an

attempt to supply quantitative support for these observations the following pertinent features were recorded during the experiments: day on which each host stopped feeding, the total number of days the host did not feed if it still survived to the end of the tenth day, the day on which each hydra produced its last bud, and the day of death (disintegration) of the host. These data, represented by averages ± the standard error for each treatment combination, are given in Table III. Not all replicates entered into the computation of the averages; the value in brackets indicates the pertinent number of replicates. These data apply to the

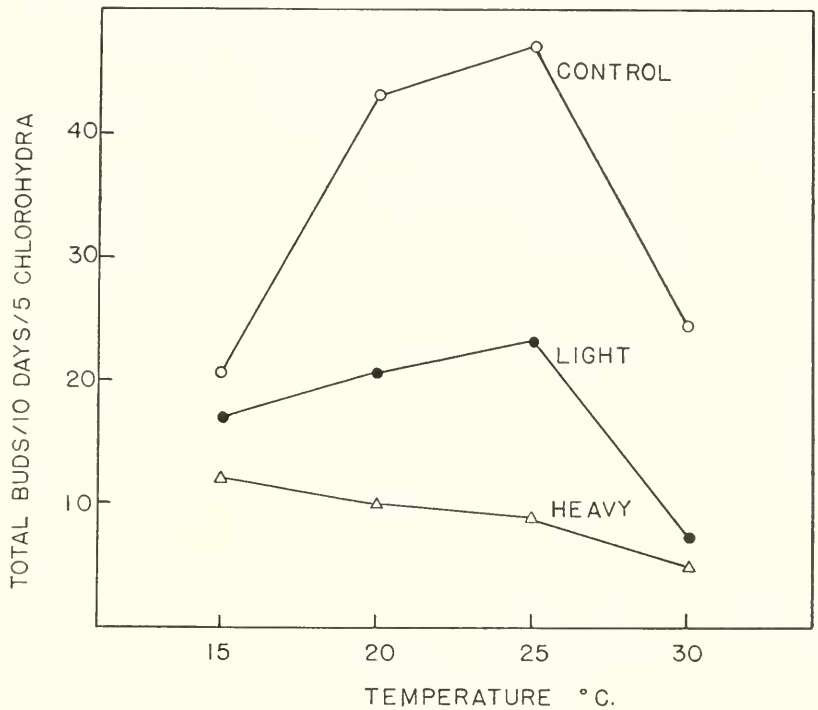

FIGURE 4. Illustration of the total number of buds produced in 10 days by groups of 5 *C. viridissima* under the various levels of temperature and initial infection. This depicts the significant interaction between temperature and infection and shows the lack of independence between these two factors.

light and heavy infection categories, since the hydras of the control group continued to feed and produce buds until the termination of the experiment. At 15° C. and at either level of infection, the hosts also continued to feed and produce buds throughout the experiment. In fact, at the light initial infection level three of the five hosts had lost the infection by the tenth day. It appears that at 15° C., *C. viridissima* is able to survive the attack of the hydramoebae. The data of Table III indicate that as temperature increases from 20° to 30°, there is a corresponding decrease in the length of time infected hosts feed, a decrease in the period of budding, and a reduction in their length of life. These results are consistent for both levels of initial infection.

Both the day the last bud was produced and the day of death of the host appear to be correlated with the day the host stopped feeding. Correlation coefficients were computed from the original data summing over all levels of temperature, excluding, of course, the 15°-level. In the first relationship $r = 0.67$ and in the latter $r = 0.78$ (both values are significant at $P < 0.01$), indicating a higher degree of correlation between the day the host stopped feeding and its eventual death. In this latter correlation, the regression coefficient is 1.23, meaning that if the host is able to feed 1.23 days longer, it can survive one more day, on the average. The regression coefficient of 0.68 in the first relationship means that an extra 0.68 day of feeding would yield an extra day of budding, on the average. Both regression coefficients are significant at $P < 0.05$.

Finally, considering budding rate in the control group, analysis of variance indicates that the mean number of buds produced in 10 days was significantly different among the levels of temperature (see Figure 4 also). Comparing each treatment mean with every other treatment by Duncan's multiple range test at the 5% level (see Steel and Torrie, 1960, p. 107) yielded the following result.

Temperature:	15°	30°	20°	25°
Mean no. buds/hydra/10 days:	4.2	4.8	8.6	9.4

The lines indicate that any two means underscored by the same line are not significantly different. Therefore, no difference in bud production exists between 15° and 30°, or between 20° and 25°. Differences do exist, however between 15° and 20°, 15° and 25°, 30° and 20°, and between 30° and 25°.

Discussion

The effect of parasitism on individual hosts may take various forms, but what is frequently encountered is an inhibition or eventual destruction of the reproductive capacities of the host. Either can alter the population sex ratio, age structure, or be detrimental to the very survival of the population if the condition should persist for a substantial length of time. For example, Yoshida (1952) found that parasitic isopods which occupy the branchial chambers of the shrimp, *Leander*, inhibit the development of sexual activity in the shrimp. The cessation of production of normal gametes in crabs parasitized by the barnacle, *Sacculina*, is a well known example. In addition, larvae of strepsipteran insects frequently cause sterility in their homopteran hosts. Cheng and Snyder (1962) also cite several cases of the effect of larval trematodes on the gonads of several snail species. Nobel and Nobel (1961), in discussing more examples, point out that the mechanisms proposed to explain these effects on reproduction include the production of toxic substances, nutritional disturbances, hormone changes, and direct mechanical destruction of gonads.

The mechanism involved in the inhibition of asexual reproduction in hydra by the parasitic hydramoeba seems to be rather simple. It appears that hydramoebae first consume the tentacles of their host, thereby rendering them incapable of feeding. Since budding rate in hydra is in part a function of its nutritional state (Loomis, 1954; Stiven, 1962b), the obvious result of the attack of the hydramoebae

is an inhibition and/or cessation of budding due to starvation, followed by the eventual death of the host.

The degree to which budding rate is inhibited, however, appears to be dependent upon the size of the initial infection. The size establishes the time at which the tentacles of the host are completely consumed, thus destroying the food-capturing ability of the host, and thereby inhibiting the host from continued cell proliferation and the formation of new buds. This conclusion is borne out by the significant correlation between the time the host stops budding and the day on which it stops feeding. Correlation coefficients, of course, measure only the degree of association between two variables and do not necessarily provide a cause and effect relationship. This must be decided by an examination of the biology of the relationship, which in this case, seems to provide a reasonable explanation for the high degree of correlation between cessation of feeding and cessation of budding.

The effect of the important environmental factor, temperature, was not independent of the action of initial parasitic infection beyond the first two cumulative days of the experiment. Figure 4 illustrates this for the 10-day period. For example, an increase of 5° (15° to 20° C.) increases bud production in the control group from 21 to 43, or 22 buds per five green hydras per 10 days. In the lightly infected group, the corresponding increase amounts to only four buds, whereas in the heavily infected group, a decrease of two buds occurs. From 25° to 30° C. decreases in total bud production occur at all levels of infection, but again the decreases are different. It is these differences that contribute to the significant interaction between temperature and infection. It must be emphasized also that the response of this host-parasite system, viewed through the interaction of these two factors, is dependent upon time. As indicated earlier, the interaction is not significant during the first two cumulative days of the experiment. Here the lines representing bud production between any two levels of temperature would be essentially parallel for the three levels of infection (*cf.* Fig. 4).

The importance of these results to experimental studies on the spread of the hydramoeba infection and to studies on factors contributing to declines in natural populations of hydra is probably best reflected in the response of the system to temperature. On the basis of the results reported here, it is postulated that at 15° C. an infected population of *C. viridissima* either eventually loses the infection and survives, other factors being favorable, or acquires some balance between the effects of the pathogenic hydramoebae and the reproductive capacity and survival of the host. In this latter case both host and parasite survive. In fact, since *Hydramoeba hydroxena* does not appear to have a viable cyst (Beers, 1963), such a relationship would constitute a reservoir of parasites. Long-term experiments are required before much credence can be given to this hypothesis.

As temperature increases and approaches 25° C., the pathogenic effects of the hydramoeba and the reproductive rate of the host both increase, but an instability develops in favor of the parasite, resulting in a cessation of budding and an eventual elimination of the host. Thus, the parasite population also succumbs. It is known that a temperature of 25° C. is very favorable for a rapid spread of the epidemic (Stiven, in press) and a subsequent rapid decline in the host

population through death. The rapid elimination of the hydra population described by Bryden (1952) perhaps exemplifies this situation. Actually, temperatures around 30° C. are frequently encountered in surface waters of southern lakes in the late summer period (Weiss and Oglesby, 1962; Bryden, 1952), and these, combined with heavy infections of hydramoebae, would lead to an almost immediate cessation of budding and to a rapid death of the host. These high temperatures also lead to the production of large numbers of hydramoebae and probably explain their great abundance in late summer in Douglas Lake, as reported by Miller (1936).

Summary

1. In prior studies on the spread of the parasite *Hydramoeba hydroxena* through populations of hydra, the density of host populations was kept fixed by removing buds and not allowing them to accumulate. Experimental analyses were undertaken, therefore, to determine the effect of this parasite on asexual reproduction in *Chlorohydra viridissima* under a range of temperatures from 15° to 30° C.

2. Budding rates were significantly different among light, heavy, and no (control) initial infections, and among four levels of temperature. The relationship between budding rate and temperature for the control group was curvilinear, with the highest rate occurring around 20° to 25° and the lowest around 15° and 30° C.

3. When the cumulative number of buds produced by the host was considered over the 10-day span of the experiments, it was found that temperature and initial infection were not independent of one another. This lack of independence was due to the fact that the difference in bud production among the levels of infection was not the same for the levels of temperature. The converse of this latter statement is also true.

4. *Hydra pseudoligactis,* which is less resistant to the attack of the hydramoebae, ceases budding almost immediately after infection.

5. In *C. viridissima*, significant correlations exist between the time the host stopped feeding and the day the host stopped budding, as well as between the time the host stopped feeding and the day it died from the attack of the hydramoebae. In addition, increases in temperature caused infected hosts to cease feeding sooner, a decrease in their period of budding, and a reduction in their length of life.

6. It appears that the destruction of the tentacles of the host by the hydramoebae renders the host incapable of feeding, and leads in turn to starvation and cessation of budding. The time at which the tentacles are rendered useless is dependent upon temperature and the size of the initial infection.

7. *C. viridissima* can tolerate the parasitic infection and continue budding at a lower temperature (*i.e.*, 15° C.). It is postulated that this balanced relationship may be a mechanism for providing a reservoir for *Hydramoeba hydroxena* in the absence of a viable cyst. However, this balance does not always occur at this temperature, since the infection is frequently lost. Long-term experiments are required before much weight can be given to this hypothesis.

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